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ORIGINAL REPORT

RUNX1 Mutations in Acute Myeloid Leukemia: Results From a Comprehensive Genetic and Clinical Analysis From the AML Study Group

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ABSTRACT

Purpose
To evaluate frequency, biologic features, and clinical relevance of *RUNX1* mutations in acute myeloid leukemia (AML).

Patients and Methods
Diagnostic samples from 945 patients (age 18 to 60 years) were analyzed for *RUNX1* mutations. In a subset of cases (n = 269), microarray gene expression analysis was performed.

Results
Fifty-nine *RUNX1* mutations were identified in 53 (5.6%) of 945 cases, predominantly in exons 3 (n = 11), 4 (n = 10), and 8 (n = 23). *RUNX1* mutations clustered in the intermediate-risk cytogenetic group (46 of 640, 7.2%; cytogenetically normal, 34 of 538, 6.3%), whereas they were less frequent in adverse-risk cytogenetics (five of 109, 4.6%) and absent in core-binding-factor AML (0 of 77) and acute promyelocytic leukemia (0 of 61). *RUNX1* mutations were associated with *MLL*-partial tandem duplications ($P = .0007$) and *IDH1*/*IDH2* mutations ($P = .03$), inversely correlated with *NPM1* ($P < .0001$), and in trend with *CEBPA* ($P = .10$) mutations. *RUNX1* mutations were characterized by a distinct gene expression pattern; this *RUNX1* mutation-derived signature was not exclusive for the mutation, but also included mostly adverse-risk AML [eg, 7q-, -7, inv(3), or t(3;3)]. *RUNX1* mutations predicted for resistance to chemotherapy (rates of refractory disease 30% and 19%, $P = .047$, for *RUNX1*-mutated and wild-type patients, respectively), as well as inferior event-free survival (EFS; $P < .0001$), relapse-free survival (RFS, $P = .022$), and overall survival ($P = .051$). In multivariable analysis, *RUNX1* mutations were an independent prognostic marker for shorter EFS ($P = .007$). Explorative subgroup analysis revealed that allogeneic hematopoietic stem-cell transplantation had a favorable impact on RFS in *RUNX1*-mutated patients ($P < .0001$).

Conclusion
AML with *RUNX1* mutations are characterized by distinct genetic properties and are associated with resistance to therapy and inferior outcome.

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INTRODUCTION

The identification of mutations—for example, in the nucleophosmin 1 (*NPM1*) gene, the fms-related tyrosine kinase 3 (*FLT3*) gene, the CCAAT/enhancer binding protein alpha (*CEBPA*) gene, the myeloid-lymphoid or mixed-lineage leukemia (*MLL*) gene, the neuroblastoma RAS viral oncogene homolog (*NRAS*) gene, the Wilms tumor (*WT1*) gene, and most recently the isocitrate dehydrogenase 1 (*IDH1*) and 2 (*IDH2*) genes in acute myeloid leukemia (AML)—has significantly improved our understanding of leukemogenesis.[1-8] Some of these mutations have been shown not only to provide important prognostic information, but also to represent potential targets for molecular therapies.[9,10] In addition, by the application of genome-wide gene expression profiling (GEP), gene signatures have been identified that separate AML into previously unrecognized biologic and/or prognostic subsets.[11-13]

The runt-related transcription factor 1 (*RUNX1*) gene is another candidate targeted by chromosomal rearrangements or intragenic mutations in acute leukemia.[14-22] Recurrent translocations involving *RUNX1* include t(8;21)(q22;

q22); *RUNX1-RUNX1T1* or t(3;21)(q26.2;q22); *EVI1-RUNX1* in AML, and t(12;21)(p13;q22); *ETV6-RUNX1* in childhood acute lymphoblastic leukemia.[23] Somatic mutations clustering within the Runt domain of *RUNX1* have been described in myelodysplastic syndrome and AML.[16-20] Of note, inherited mutations of *RUNX1* were identified as a cause of the autosomal familial platelet disorder that predisposes to the development of MDS and AML.[24,25]

In initial studies, *RUNX1* mutations have been associated with undifferentiated morphology (French-American-British [FAB] M0) and with specific chromosome aberrations, such as trisomy 21 and trisomy 13.[17,18,21] In a recent study of 156 cases with AML, highly selected for specific FAB and cytogenetic subgroups,[21] *RUNX1* mutations were detected in almost half (46%) of FAB M0 cases and in 80% of cases exhibiting trisomy 13. So far, only one study has been published reporting frequency and clinical significance of *RUNX1* mutations in an unbiased cohort of patients with AML (15 to 90 years of age).[22] In this study of 470 Taiwanese adult patients with AML, *RUNX1* mutations were found in 13.2% of patients. Mutations were associated with a lower complete remission (CR) rate and with inferior disease-free survival (DFS) and overall survival (OS).

Runx family proteins were found to have an essential role in the regulation of gene expression by, for example, temporal transcriptional repression and epigenetic silencing via chromatin alterations, especially in the context of chromosomal translocations.[26] These findings might have therapeutic implications as the *RUNX1*-associated gene deregulation and hematopoietic differentiation block might be effectively targeted by epigenetic therapeutic approaches.

The objectives of our study were to evaluate the frequency and clinical impact of *RUNX1* mutations in the context of cytogenetic and molecular genetic markers in a large cohort of younger adult patients with AML entered onto prospective clinical trials of the German-Austrian AML Study Group (AMLSG). To gain further insights into the biology of *RUNX1*-mutated AML, GEP was performed in a subset of cases.

PATIENTS AND METHODS

Patient Samples

Diagnostic bone marrow (BM) or peripheral blood (PB) samples from 945 patients with AML (age 18 to 60 years) were analyzed. Patients were enrolled onto two consecutive AMLSG multicenter treatment trials: AML HD98A (n = 651; NCT00146120) and AMLSG 07-04 (n = 294; NCT00151242). Treatment details of the protocols are given in the Appendix (online only). Patients with acute promyelocytic leukemia (APL) were treated in the APL HD 95 trial.[27] All patients gave informed consent for treatment and genetic analysis according to the Declaration of Helsinki.

Cases were molecularly studied for the presence of the recurring gene fusions *RUNX1-RUNX1T1*, *CBFB-MYH11*, *MLL-MLLT3*, and *PML-RARA* (either by fluorescence in situ hybridization or polymerase chain reaction [PCR]) and for gene mutations in *FLT3* (internal tandem duplications [ITD] and tyrosine kinase domain [TKD] mutations at codons D835 and I836); *NPM1*; *MLL* (partial tandem duplication [PTD]); *IDH1* and *IDH2* (analyses restricted to the AML HD98A trial, n = 642); and *CEBPA*, *NRAS*, and *WT1* (analyses of the latter three in cytogenetically normal [CN] AML only) genes.[6,28]

Analysis of RUNX1 Mutations

The entire coding region of *RUNX1* (exons 1 through 8) was amplified by PCR using intron-exon flanking primer pairs and subjected to direct sequencing according to standard protocols (Appendix Table A1; online only). PCR reactions and sequencing analyses were repeated in all cases showing sequence variations.

In addition, PB samples from 29 healthy volunteers were analyzed for the presence of *RUNX1* polymorphisms. In 18 of the 53 mutated AML cases, germline material (DNA obtained from buccal swabs or from BM in CR) was studied for the presence of *RUNX1* germline mutations. Finally, all *RUNX1* sequence variations were aligned to different single nucleotide polymorphism databases[29-31] to detect known polymorphisms.

GEP Profiling

For a subset of AML HD98A cases with available high-quality RNA (n = 269), GEP was performed as previously described using cDNA microarrays.[32] The complete GEP data set is also accessible at gene expression omnibus (GEO accession GSE23312). For data analysis, fluorescence ratios were normalized as described,[32] and for all subsequent analyses, only differentially expressed clones were included.

Statistical Analyses

The statistical analyses for GEP as well as for the clinical outcome analyses are provided in the Appendix.[33-41]

RESULTS

Frequency and Types of RUNX1 Mutations

Fifty-nine *RUNX1* mutations were identified in 53 (5.6%) of 945 cases (Appendix Table A2; online only). Mutations clustered in exon 3 (n = 11), 4 (n = 10), and 8 (n = 23), but also occurred in exon 5 (n = 5), 6 (n = 7), and 7 (n = 3). There were six cases with two concurrent *RUNX1* mutations. Most of the mutations were frameshift mutations as a result of insertions or deletions (n = 44), but in 15 cases we also detected single nucleotide substitutions (two nonsense, one silent, 12 missense mutations). All mutations were heterozygous. There were 26 mutations in 21 patients within exons 3 to 5; among them 14 were predicted to lose the DNA-binding domain RHD because of generating a stop codon. Regarding the mutations of exons 6 to 8 involving the transcriptional activation domain, 21 of 33 mutations in 31 patients might result in a truncated RUNX1 protein.

In 23 patients with AML, we found a sequence variation at amino acid codon 56 (L56S) in exon 3 that was recently described as polymorphism.[42] This polymorphism was not detected in 29 healthy volunteers, but occurred in nine of 18 germline samples that have been tested. In contrast, in none of the 18 germline samples the respective gene function altering mutation was found.

Association of RUNX1 Mutations With Clinical Characteristics, Cytogenetics, and Other Molecular Markers

RUNX1 mutations were mainly found in the cytogenetic intermediate-risk group (46 of 640, 7.2%), most commonly in CN-AML (34 of 538, 6.3%) and in AML with trisomy 8 within a noncomplex karyotype (five of 36, 14%; Table 1); the frequency in the high-risk group was lower (five of 109, 4.6%), and no mutations were found in core-binding-factor AML (0 and 77) and APL (0 and 61). Because APL did not exhibit *RUNX1* mutations and patients were treated differently, patients with APL were excluded from further analyses.

RUNX1 mutations were associated with the presence of *MLL*-PTD ($P = .0007$) and *IDH1/IDH2* mutations ($P = .03$; $IDH1^{R132}$, n = 7; $IDH2^{R140}$, n = 4; $IDH2^{R172}$, n = 1), but inversely correlated with *NPM1* mutations ($P < .0001$). No significant associations were

Table 1. Pretreatment Clinical Characteristics, Cytogenetic Risk Group Assignment, Molecular Features, and Outcomes According to *RUNX1* Mutational Status in all AML (APL excluded)

Characteristic	*RUNX1* Mutated (n = 53)		*RUNX1* Wild-Type (n = 831)		*P*
	No.	%	No.	%	
Age, years					.42
Median	48.1		48.2		
Range	19-60		18-60		
Male sex	31	59	442	53	.40
AML history					.10
De novo	45	85	767	92	
Secondary	5	9	27	3	
Therapy related	3	6	32	4	
WBC count, $\times 10^9$/L					.41
Median	13.4		17.6		
Range	0.9-235.0		0.2-427.0		
Platelet count, $\times 10^9$/L					.99
Median	51		56		
Range	6-242		2-746		
Hemoglobin, g/dL					.64
Median	8.95		8.90		
Range	4.8-13.6		3.0-17.6		
Blood blasts, %					.69
Median	41.5		40		
Range	0-98		0-100		
Bone marrow blasts, %					.34
Median	80		74		
Range	20-100		2-100		
FAB classification*					
M0	5	16	28	6	.04
M1	7	23	85	17	.47
M2	4	13	157	32	.03
M4	8	26	135	27	1.0
M5	6	35	63	13	.28
M6	1	2	13	3	.58
M7	0	0	10	2	1.0
Missing	9		59		
Cytogenetic risk group					.02
Favorable	0	0	77	9	
Intermediate	46	87	594	72	
Normal karyotype	34	64	504	61	
Adverse	5	9	104	13	
Missing	2		56		
Specific cytogenetic abnormalities					
Monosomy 7†	0		36		
Trisomy 8‡	5		31		
Trisomy 13§	1		4		
Trisomy 13‡	1		3		
Trisomy 21§	1		8		
Trisomy 21‡	0		8		
Abnl(12p) †	3		18		
NPM1					< .001
Wild-type	48	91	515	63	
Mutated	5	9	307	37	
FLT3-ITD					.87
Absent	39	75	593	73	
Present	13	25	216	27	
FLT3-TKD					.42
Wild-type	47	96	704	91	
Mutated	2	4	68	9	

(continued on following page)

Table 1. Pretreatment Clinical Characteristics, Cytogenetic Risk Group Assignment, Molecular Features, and Outcomes According to *RUNX1* Mutational Status in all AML (APL excluded) (continued)

Characteristic	*RUNX1* Mutated (n = 53)		*RUNX1* Wild-Type (n = 831)		*P*
	No.	%	No.	%	
MLL-PTD					.0007
Absent	37	75	687	92	
Present	12	25	60	8	
*IDH1/2**					.03
Wild-type	26	68	452	83	
Mutated	12	32	92	17	
CEBPA (CN-AML)					.10
Wild-type	32	97	396	86	
Mutated	1	3	64	14	
Monoallelic	1	3	22	5	
Biallelic	0		42	9	
WT1 (CN-AML)					.55
Wild-type	20	83	332	87	
Mutated	4	17	51	13	
NRAS (CN-AML)					.60
Wild-type	23	82	356	85	
Mutated	5	18	63	15	

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; FAB, French-American-British; ITD, internal tandem duplication; TKD, tyrosine kinase domain; PTD, partial tandem duplication; CN, cytogenetically normal.
*Analysis is restricted to AML HD98A trial.
†All cases, excluding core-binding factor leukemias.
‡All cases, excluding core-binding factor leukemias within noncomplex karyotype.
§All cases, excluding core-binding factor leukemias, within complex karyotype.

found between *RUNX1* and *FLT3*-TKD mutations ($P = .42$) or *FLT3*-ITD ($P = .87$) (Fig 1). Of note, presence of *FLT3*-ITD and *IDH* mutations concurrently with *RUNX1* mutations were mutually exclusive. Among CN-AML, *RUNX1* mutations in trend negatively correlated with *CEBPA* mutations ($P = .10$); the single case of *CEBPA* mutation had monoallelic mutation. There was no correlation with *WT1* ($P = .55$) or *NRAS* ($P = .60$) mutations.

Regarding presenting clinical characteristics, *RUNX1* mutations were associated with FAB M0 ($P = .04$) and less frequently with FAB M2 ($P = .03$) morphology (Table 1). There was no significant difference in clinical characteristics according to exon involvement of the mutations (exons 3 to 5 *v* exons 6 to 8) except for BM blasts ($P = .071$; Appendix Table A3; online only).

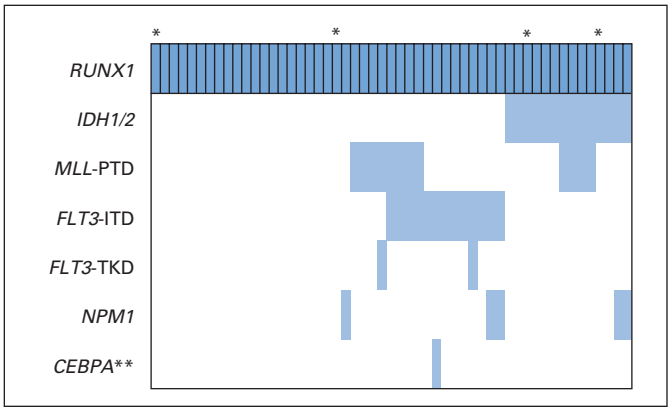

Fig 1. Frequencies and distribution of the mutations of *IDH1/2*, *MLL*-PTD, *FLT3*, *NPM1*, and *CEBPA* in the *RUNX1*-mutated patients. Each *RUNX1*-mutated patient is represented by a blue column. PTD, partial tandem duplication; ITD, internal tandem duplication; TKD, mutation of the tyrosine kinase domain. *Missing mutation status of *MLL*-PTD; **one case with monoallelic *CEBPA* mutation.

Response to Induction Therapy

For correlation with clinical outcome, 878 non-APL AML cases (missing follow-up data, n = 6) were considered. *RUNX1* mutations were associated with resistance to chemotherapy. Response to induction therapy was as follows: CR, 60.4% (32 of 53) and 73.4% (606 of 825; $P = .055$); refractory disease (RD), 30% and 19% ($P = .047$); early/hypoplastic death, 9% and 8% ($P = .61$) for *RUNX1*-mutated and *RUNX1*-wildtype AML, respectively. For the subgroup of CN-AML, there was in trend a difference between *RUNX1*-mutated and *RUNX1*-wild-type AML regarding CR rate (22 of 34 *v* 388 of 499; $P = .09$) and a significant difference in RD (10 of 32 *v* 77 of 499; $P = .05$).

In multivariable analysis, *RUNX1* mutation did not significantly impact achievement of CR, neither in the entire cohort nor in the subsets of intermediate-risk or CN-AML (data not shown). Significant variables for achievement of CR in the entire cohort were log WBC, age, *FLT3*-ITD, cytogenetic risk group, and *NPM1* mutations.

Survival Analysis

The median follow-up time for survival in the 878 non-APL patients with AML was 4.5 years (95% CI, 4.3 to 5.0); the estimated 4-year RFS and OS of the entire cohort were 43% (95% CI, 39% to 47%) and 44% (95% CI, 41% to 48%), respectively.

RUNX1 mutations were associated with inferior survival, the 4-year estimated survival rates for *RUNX1*-mutated and *RUNX1* wild-type patients were as follows: EFS, 8% versus 30% (log-rank,

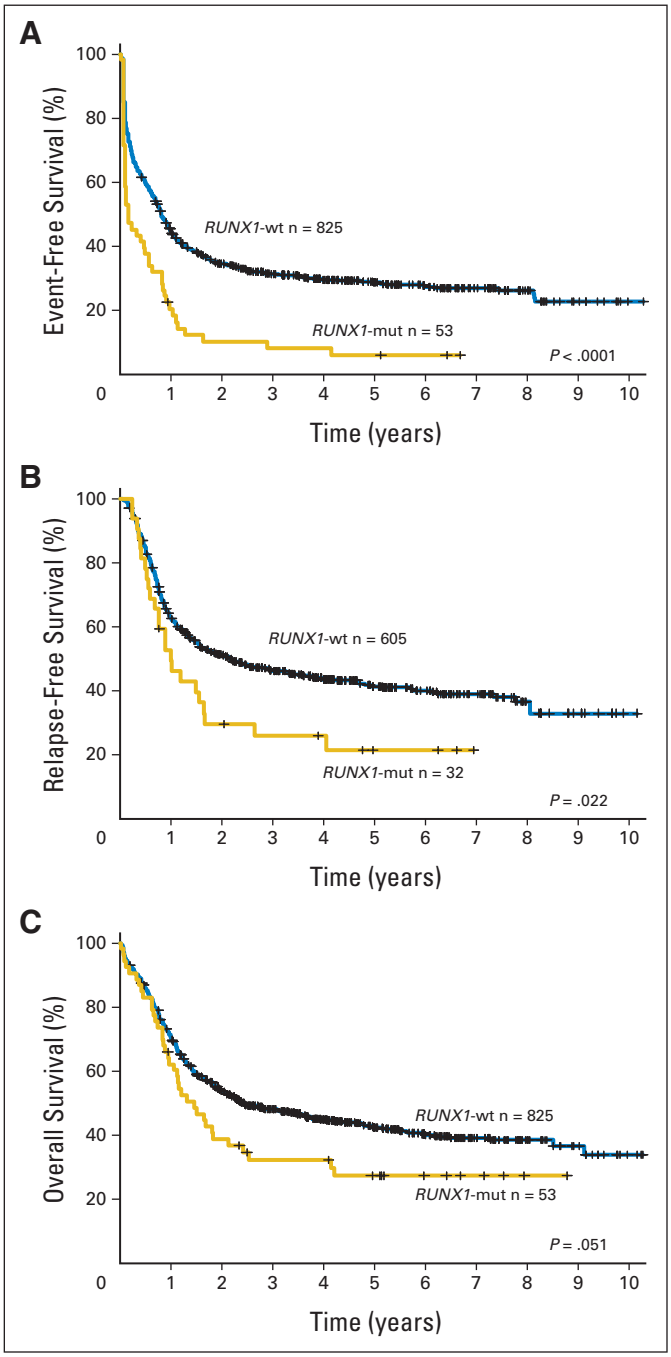

Fig 2. Kaplan-Meier survival estimates, according to *RUNX1* mutation status. Data are shown for (A) event-free survival, (B) relapse-free survival, and (C) overall survival. wt, wild type; mut, mutated.

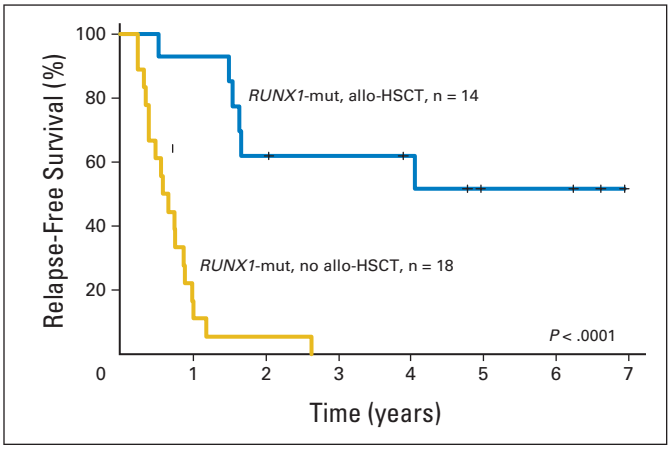

Fig 3. Kaplan-Meier survival estimates for relapse-free survival for all patients according to allogeneic hematopoietic stem-cell transplantation (allo-HSCT). Data are shown for *RUNX1*-mutated patients in whom allo-HSCT in first complete remission was performed or not. mut, mutated.

$P < .0001$); RFS, 26% versus 44% ($P = .022$); and OS, 32% versus 45% ($P = .051$), respectively (Fig 2). Among patients with CN-AML, *RUNX1* mutations predicted for inferior EFS, but no difference was found for RFS and OS: 4-year EFS, 10% versus 34% (log-rank, $P < .0001$); RFS, 29% versus 43% ($P = .21$); OS, 39% versus 50% ($P = .09$) for *RUNX1*-mutated and *RUNX1* wild-type patients, respectively.

Allogeneic hematopoietic stem-cell transplantation (HSCT) in first CR was performed in 14 (44%) of 32 *RUNX1*-mutated patients. Of note, all 18 patients receiving repetitive cycles of high-dose cytarabine or autologous HSCT had an event in RFS (events: relapse, n = 17; death, n = 1), whereas in patients receiving allogeneic HSCT, eight of 14 were event-free (events: relapse, n = 2; death, n = 4). This translated into a significantly better RFS compared with the patients receiving conventional intensive postremission therapy (4-year RFS of 0% and 52% [log-rank, $P < .0001$], respectively; Fig 3). Of note, there was no significant difference in pretreatment patient characteristics between these two groups (Appendix Table A4, online only).

In multivariable analyses performed for all patients as well as for the subgroups of patients with intermediate-risk AML or CN-AML, *RUNX1* mutation was a significant marker for inferior EFS in the entire cohort (hazard ratio [HR], 1.494; $P = .011$) and in the intermediate-risk group (HR, 1.607; $P = .005$), but not in CN-AML (data not shown); there was no significant impact on RFS and OS (for the entire cohort see Table 2). However, in multivariable analyses of RFS censoring for allogeneic HSCT in first CR at the date of transplantation, *RUNX1* mutation again was a significant adverse factor (HR, 1.70; $P = .04$).

Finally, we performed explorative subgroup analyses in *RUNX1*-mutated AML to evaluate the impact of additional *FLT3*-ITD, *MLL*-PTD, and *IDH1* and *IDH2* mutations. AML with *RUNX1* mutation and concurrent *FLT3*-ITD had an in trend inferior OS as compared with those without *FLT3*-ITD (18% *v* 37%; log-rank, $P = .07$). *MLL*-PTD and *IDH* mutations had no impact.

RUNX1 *Mutation Cases Are Associated With a Distinct Gene Expression Pattern*

Significance analysis of microarrays (SAM) revealed 148 genes differentially expressed between *RUNX1*-mutated (n = 14) and *RUNX1* wild-type cases (n = 255; false discovery rate, = 0.09; Data Supplement). Hierarchical cluster analysis based on the SAM-derived *RUNX1* mutation-associated signature (average linkage clustering; similarity metric: correlation, uncentered) showed that all 14 *RUNX1*-mutated cases were in part characterized by the respective genes as they all grouped together in one cluster (Data Supplement). However, the deregulated gene pattern was not exclusive for *RUNX1*-mutated AML, with other AML cases also displaying a *RUNX1*-mutated–like

Table 2. Multivariable Analyses for All Patients With AML (APL excluded): Cox Regression Model on EFS, RFS, and OS Performed for the Entire Cohort

End Point and Variable	HR	95% CI	*P*
EFS			
RUNX1 mutation	1.494	1.096 to 2.038	.011
Type of AML*	1.043	0.777 to 1.400	.778
Log_{10}WBC	1.250	1.089 to 1.435	.002
Log_{10}platelet	1.003	0.810 to 1.243	.975
MLL-PTD	0.907	0.680 to 1.211	.508
Age	1.018	1.009 to 1.027	< .001
FLT3-ITD	1.583	1.297 to 1.932	< .001
NPM1 mutation	0.510	0.414 to 0.628	< .001
FLT3-TKD mutation	0.883	0.637 to 1.223	.453
BM blasts	1.000	0.998 to 1.002	.980
Cytogenetic adverse risk	2.226	1.733 to 2.859	< .001
Cytogenetic low risk	0.472	0.333 to 0.670	< .001
RFS			
RUNX1 mutation	1.296	0.843 to 1.992	.237
Type of AML*	1.376	0.911 to 2.079	.129
Log_{10}WBC	1.459	1.203 to 1.770	< .001
Log_{10}platelet	0.866	0.650 to 1.155	.328
MLL-PTD	1.028	0.703 to 1.504	.887
Age	1.023	1.011 to 1.035	< .001
FLT3-ITD	1.773	1.380 to 2.278	< .001
NPM1 mutation	0.611	0.471 to 0.793	< .001
FLT3-TKD mutation	0.978	0.646 to 1.481	.917
BM blasts	1.000	0.995 to 1.004	.844
Cytogenetic adverse risk	2.112	1.459 to 3.058	< .001
Cytogenetic low risk	0.600	0.397 to 0.908	.016
RFS (allo-HSCT censored)			
RUNX1 mutation	1.712	1.027 to 2.853	.039
Type of AML*	2.086	1.228 to 3.543	.007
Log_{10}WBC	1.416	1.129 to 1.777	.002
Log_{10}platelet	0.868	0.616 to 1.223	.419
MLL-PTD	1.239	0.777 to 1.978	.368
Age	1.016	1.003 to 1.031	.020
FLT3-ITD	2.040	1.505 to 2.763	< .001
NPM1 mutation	0.540	0.394 to 0.740	< .001
FLT3-TKD mutation	0.971	0.613 to 1.539	.902
BM blasts	1.001	0.996 to 1.007	.717
Cytogenetic adverse risk	1.372	0.790 to 2.384	.262
Cytogenetic low risk	0.543	0.349 to 0.846	.007
OS			
RUNX1 mutation	1.077	0.755 to 1.535	.684
Type of AML*	1.448	1.059 to 1.979	.020
Log_{10}WBC	1.448	1.238 to 1.693	< .001
Log_{10}platelet	0.931	0.736 to 1.177	.551
MLL-PTD	1.032	0.748 to 1.424	.847
Age	1.038	1.027 to 1.048	< .001
FLT3-ITD	1.751	1.406 to 2.181	< .001
NPM1 mutation	0.752	0.595 to 0.950	.017
FLT3-TKD mutation	0.756	0.516 to 1.107	.150
BM blasts	1.000	0.998 to 1.002	.682
Cytogenetic adverse risk	2.591	1.988 to 3.376	< .001
Cytogenetic low risk	0.463	0.301 to 0.713	.004

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; EFS, event-free survival; RFS, relapse-free survival; OS, overall survival; HR, hazard ratio; PTD, partial tandem duplication; ITD, internal tandem duplication; TKD, tyrosine kinase domain; BM, bone marrow; allo-HSCT, allogeneic hematopoietic stem-cell transplantation.
*Type of AML: de novo AML, secondary AML, or treatment-related AML.

pattern (Data Supplement). Interestingly, the respective cluster was enriched for cytogenetic adverse-risk groups like cases with deletion 7q/monosomy 7 [del(7q)/-7], inv(3)/t(3,3) involving the *EVI1* gene, and AML cases with complex karyotypes, whereas the *RUNX1* wild-type cluster (cluster 2) contained almost all cases of inv(16), t(8;21), t(15;17), and t(11q23) ($P < .0001$, χ^2 test; Figs 4A to 4C). In accordance, the inverse association of *RUNX1* and *NPM1* mutations was also reflected at the gene expression level as the *RUNX1*-mutated/*RUNX1*-mutated–like cases were inversely correlated with *NPM1* mutations ($P < .0001$, Fisher's exact test).

To account for the cytogenetic and molecular heterogeneity of the *RUNX1*-mutated cases, we also performed a subgroup analysis within the *IDH*-mutated AML cases, which provided similar results as the SAM analysis across all cases (Data Supplementary). Furthermore, although the *RUNX1* mutation-associated signature was enriched for genes belonging to pathways associated with, for example, tumor necrosis factor signaling and apoptosis (Data Supplement), there was also a considerable overlap with pathways enriched in putative *RUNX1*-associated gene lists (Molecular Signatures Database MSigDBK, Data Supplement).

DISCUSSION

In our study of 945 unselected younger adult patients with AML derived from prospective multicenter treatment trials, *RUNX1* mutations were detected with an overall incidence of 5.6%. Mutations were associated with specific clinical and genetic characteristics and predicted for inferior survival.

The frequency of mutations was somewhat lower compared with the study by Tang et al[22] (62 of 470, 13.2%) that is so far the only published study in a larger AML cohort. In part, this may be explained by patient selection; that is, in the Taiwanese study, all adult patients (15 to 90 years of age) were included, and patients with *RUNX1* mutations were significantly older compared with those with wild-type *RUNX1* (62 *v* 48 years, $P = .01$). In addition, in the Taiwanese study, a male predominance for *RUNX1*-mutated AML was described, which was not the case in our study. Besides patient selection, the racial background (white *v* Asian) may account for the observed differences in mutation frequencies and sex distribution. Similar to previous studies, we found a correlation with undifferentiated FAB M0 morphology and with intermediate-risk cytogenetics.[21] In accordance with the study by Tang et al,[22] *RUNX1* mutations were somewhat less frequent in cytogenetic high-risk AML and did not occur in CBF-AML and APL. Among intermediate-risk AML, *RUNX1* mutations were associated with normal karyotype and with trisomy 8 (occurring as sole abnormality or within a noncomplex karyotype). The previously reported association of *RUNX1* mutations with trisomy 13 could not be evaluated in our study because there was only one case with trisomy 13 as sole aberration. Analogous to the study by Dicker et al,[21] the majority of trisomy 13 cases occurring in a complex karyotype had wild-type *RUNX1*. With regard to the correlation with other molecular makers, we observed a significant correlation of *RUNX1* mutations with *MLL*-PTD and *IDH* mutations and an inverse correlation with *NPM1* and *CEBPA* mutations (Fig 1). These data suggest that *RUNX1* mutations contribute to leukemogenesis by other mechanisms than do *NPM1* and *CEBPA* mutations.

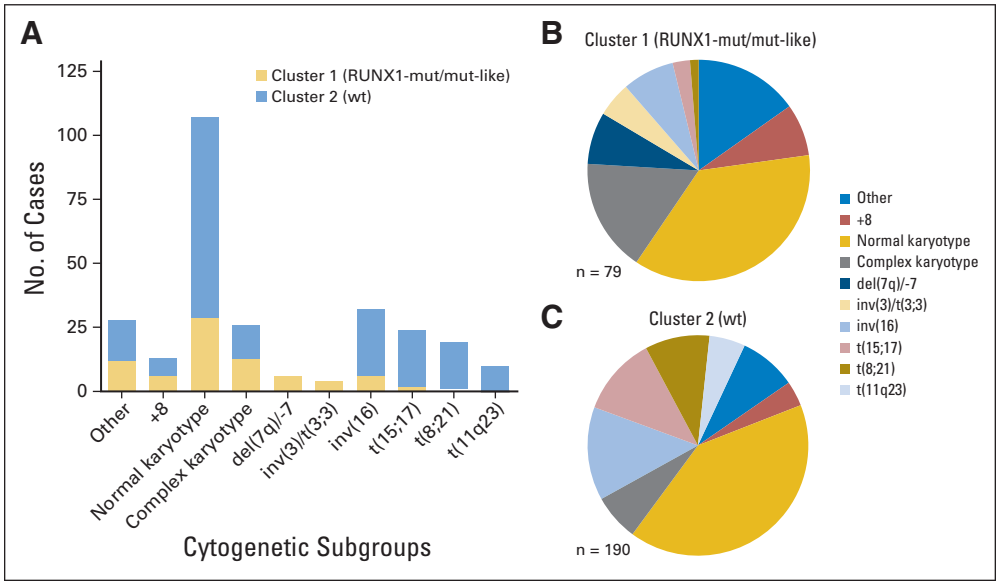

Fig 4. Hierarchical cluster analysis on the basis of the significance analysis of microarrays (SAM) –derived *RUNX1* mutation–associated signature. (A) Comparison of the distribution of cytogenetic subgroups within the hierarchical cluster analysis defined clusters (Data Supplement). Cytogenetic adverse-risk groups with deletion 7q or monosomy 7 [del(7q)/-7] and with inv(3) or t(3;3) were significantly enriched in the *RUNX1* mutation–associated cluster 1 ($P < .0001$, χ^2 test). (B and C) The proportion of AML cases with complex karyotypes (indicated in gray) was significantly higher in (B) the *RUNX1*-mutation–associated cluster 1, whereas almost all inv(16), t(8;21), t(15;17), and t(11q23) cases were present in (C) the *RUNX1*-wild-type–associated cluster 2. mut, mutated; wt, wild-type.

In our study, *RUNX1* mutations were significantly associated with resistance to induction chemotherapy; rates of RD were 30% and 18% for *RUNX1*-mutated and *RUNX1* wild-type AML, respectively. In univariable analysis, *RUNX1*-mutations predicted for inferior EFS, RFS, and OS. In the subset of patients with CN-AML, *RUNX1* mutations only predicted for inferior EFS, but not for inferior RFS and OS. Explorative subset analyses showed that concurrent *FLT3*-ITD may negatively impact OS in *RUNX1*-mutated AML, whereas there was no effect of additional *MLL*-PTD or *IDH1* and *IDH2* mutations. Of note, allogeneic HSCT had a favorable impact on outcome in *RUNX1*-mutated AML. In fact, all *RUNX1*-mutated patients relapsed after conventional consolidation therapy including repetitive cycles of high-dose cytarabine and autologous HSCT, whereas the 4-year RFS after allogeneic HSCT was 52% (Fig 3). In multivariable analysis, *RUNX1* mutation was a significant factor for EFS in the entire cohort as well as in the subgroup of cytogenetically intermediate-risk patients. The effect was also significant for RFS, but not OS, if allogeneic HSCT in first CR was censored at the date of transplantation. These data are somewhat in contrast to those by Tang et al,[22] who found *RUNX1* mutations to be a significant factor also for inferior OS. In part, this difference may be explained by the fact that in that study older patients were included, and the proportion of patients receiving allogeneic HSCT was lower. Because *RUNX1* mutations occur with a relatively low incidence, it is difficult to show its prognostic impact, especially within the context of well-established strong prognostic molecular markers. In addition, the impact of allogeneic HSCT further complicated the evaluation as a prognostic marker by reducing the sample size after censoring patients who underwent transplantation.

GEP analysis also provided evidence that *RUNX1*-mutated AML shares a distinct biology that overlaps with other high-risk AML. In agreement, a recent analysis showed an enrichment of *RUNX1*-mutated cases in a cohort of AML comprising complex karyotype AML and cases with del(7q)/-7 as well as *EVI1* rearrangements.[42] Thus aberrations of *RUNX1*, *EVI1*, and yet unknown molecular mechanisms share the deregulation of identical pathways and thus might present with a similar phenotype. Here, *RUNX1* mutations seem to contribute significantly, as the respective gene pattern contained genes known to be associated with *RUNX1* aberrations in leukemia such as *BAALC*, a gene also highly expressed in t(8;21) AML,[43,44] and *MET*, a proto-oncogene that has been linked to *ETV6-RUNX1* rearrangements.[45] Finally, deregulated expression of apoptotic pathway members were another prominent feature, suggesting that altered regulation of apoptosis, exemplified by higher expression levels of *BCL2L1* (*BCL2-like 1*, also known as *Bcl-X* or *Bcl-XL*), might play an important role in the *RUNX1*-mutated subgroup.[46]

In conclusion, the findings of our study further strengthen the data that *RUNX1* mutations are characterized by distinct biologic and clinical features. In accordance, this AML subgroup shows almost no overlap with other genetic subsets (eg, defined by *NPM1* mutations). Clinically, *RUNX1* mutations predict for resistance to chemotherapy. Given the available data, *RUNX1* mutational status should not be integrated into therapeutic decision making. Further studies with a focus on intermediate-risk AML are needed to evaluate the predictive impact of *RUNX1* mutations for allogeneic HSCT.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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